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Degradation of pullulan irradiated in hydro-methanolic blends: influence of cinnamyl alcohol at low absorbed dose of radiation

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ABSTRACT. This study reports on the effects of electron beam radiation on pullulan in both the dry state and hydro-methanolic blends containing cinnamyl alcohol (CA). The radiation chemical yields of scission ($G(S)$) and crosslinking ($G(X)$) were determined using Saito's formalism applied to the evolution of molecular weight (MW) with increasing absorbed dose of radiation. To satisfy the requirements of the statistical treatment, commercially available pullulan was fractionated to obtain a monomodal MW distribution with a dispersity close to 2. The changes in MW profiles were monitored by SEC with differential refractive index and UV detection. The introduction of small quantities of CA in the pullulan blends led to a significant decrease in $G(S)$ and $G(X)$, **protecting pullulan against radiation-induced effects likely via** energy and electron transfer. In presence of larger amounts of CA, irradiation at higher dose induced an increase in molecular mass with concomitant grafting of the aromatic additive.

Keywords: Scission, Crosslinking, Pullulan, Cinnamyl Alcohol, **Radiolytic yields, Radiation protection.**

24 **1. Introduction**

25 High-energy radiation treatments applied to polysaccharides continue to arouse considerable
26 attention within the scientific community as a green method for modifying a broad class of
27 natural and renewable resources (Al-Assaf et al., 2016). The resulting structural
28 transformations are dependent not just on the induced chemical transformations at molecular
29 level. Physical interactions and molecular arrangements at the nano- and mesoscale levels can
30 have a strong influence on reactivity when complex blends are processed under high energy
31 radiation. As a result, there is a great technological potential for tailoring the properties of
32 polysaccharides (Phillips, 1962; Bhat & Karim, 2009; Braşoveanu & Nemţanu, 2018).

33 The behavior of polysaccharides submitted to ionizing radiation is roughly described by their
34 general trend towards chain scission with a degree depending primarily on the structure of the
35 polymer, on chemical treatments possibly performed prior to irradiation, and on the degree of
36 hydration (Ivanov, 1992; Bhat & Karim, 2014). Some applications aim at achieving a
37 controlled decrease of the polysaccharides molecular weight (MW) by radiation-induced
38 chain scission for biomass deconstruction (Driscoll et al., 2009; Driscoll et al., 2014) or to
39 produce chitosan oligomers (Choi et al., 2002, Hai et al., 2003). **Yet**, the degradative behavior
40 is often a limitation in developing new applications based on radiation-induced modification.

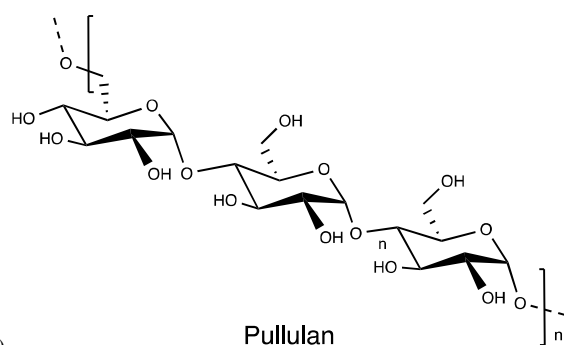
41 To overcome the radiation-induced degradation of polysaccharides while achieving the
42 desired structural and chemical modifications, alternative processing methods with corrective
43 actions have been proposed. These methods either consist of blending them with monomers
44 that polymerize and form crosslinks under ionizing radiation or convert reactive side-groups
45 with appropriate functionalities, for example, acetic or acrylic esters. Both approaches try to
46 balance chain scission with crosslinking that results in gel formation upon irradiation (Fei et
47 al. 1999; Wach et al. 2001; Ogawa et al. 2002; Wach et al. 2003; Della Giustina et al. 2019).

48 This type of treatment strongly modifies the architecture of the polysaccharide, hence its
49 properties are likely to be changed, but it is an effective way to counteract the formation of
50 low MW fragments, when this is undesirable. In the case of the radiation-induced
51 modification of starch, a major contributor to bio-based plastics with a limited environmental
52 footprint, our group has reported on original methods to mitigate the water sensitivity and to
53 reduce retrogradation of amorphized starch by blending it with lignin or with allyl urea
54 followed by electron beam (EB) processing (Olivier et al., 2000, Olivier et al. 2001; Lepifre
55 et al., 2004a, Lepifre et al., 2004b). Further studies on starch and maltodextrin including low
56 MW aromatic additives such as cinnamyl alcohol (CA) revealed a strong dependence of the
57 radiation-induced degree of modification (i) on the chemical composition of the blend, and
58 (ii) on inter-molecular associations between the constituents with formation of inclusion
59 complexes in helical domains of the glucan (Khandal et al., 2012; Khandal et al., 2013). The
60 occurrence of such supramolecular interactions does not allow for a simple interpretation of
61 the radiation-induced evolution of MW distribution and gel fraction as a function of blend
62 composition and absorbed dose of radiation (Khandal et al., 2015).

63 In this context, we wished to pursue a more basic investigation with a quantitative assessment
64 of the role and the degree of influence of aromatic additives such as CA on the competing
65 phenomena of scission and crosslinking along glucan chains, using the theoretical formalisms
66 developed by several authors (Saito, 1972; Charlesby, 1980, Moad & Winzor, 1998; Rosiak,
67 1998) to quantify the radiation chemical yields $G(S)$ and $G(X)$ for scission and for interchain
68 linking, respectively, defined as the number of occurrence for each type of event per unit
69 absorbed dose of radiation.

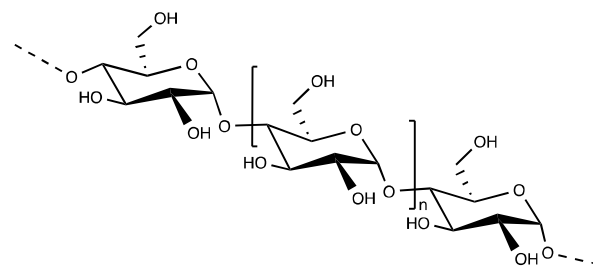
70 Since starch and starch-derived maltodextrins are multimodal mixtures of linear and branched
71 polysaccharides, generally with a broad MW distribution, they cannot be used for the

72 determination of $G(S)$ and $G(X)$. Hence, it was necessary to select an appropriate model for
73 glucans submitted to irradiation in such media, that would comply with the requirements of
74 the theoretical formalism detailed in a forthcoming section of this article. Pullulan is an α -
75 glucan constituted of 1,6-linked maltotriose units, hence including anhydroglucose units
76 (AGU) linked by α -1,4 glycosidic linkages, the basic constituting unit and connecting bonds
77 present in starch, as shown in Scheme 1.



78 a)

Pullulan

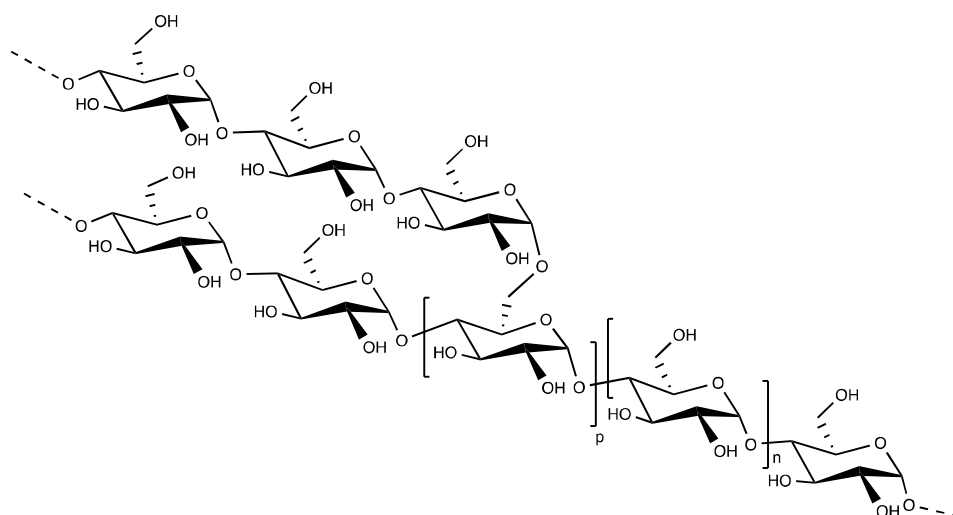


79 b)

Amylose

80

81

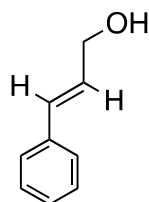


82

c)

Amylopectine

d)



83

Cinnamyl alcohol

84 Scheme 1. Molecular structure of pullulan (a), of the main components of starch, amylose (b)
 85 amylopectine (c), and of cinnamyl alcohol (CA).

86 Being a well-defined linear polysaccharide (Singh et al., 2008; Trinetta & Cutter, 2016, Singh
 87 et al. 2019), pullulan can be easily fractionated by chromatography or precipitation
 88 techniques to obtain a desirable MW distribution. Another crucial aspect is its tendency to
 89 adopt a random coil conformation in aqueous blends (Nishinari et al., 1991), rather than
 90 forming helices with hydrophobic internal cavities exhibiting strong affinity for aromatic
 91 solutes, such as CA. Hence, it can be regarded as a good model for monitoring changes in
 92 chain length and for the statistical formalisms that allow for the quantification of radiation
 93 chemical yields associated with scissioning and interchain coupling.

94 2. Experimental

95 2.1 Materials

96 Pullulan was obtained from ABCR GmbH & Co. and was fractionated for the higher
97 molecular mass portion and freeze-dried before use. Water was de-ionized using the Waters
98 Milli-Q purification system. Reagent grade methanol and cinnamyl alcohol (CA) were
99 purchased from Sigma-Aldrich and used as received.

100 *2.2 Fractionation of pullulan*

101 The commercial sample of pullulan (14 g) was dissolved in distilled water (300 mL) to obtain
102 a homogeneous solution. Methanol (200 mL) was added slowly to this solution under
103 constant magnetic stirring until it became turbid and milky. The mixture was stirred for a few
104 minutes to allow complete precipitation of the large molecular chains of the pullulan. The
105 precipitate was then allowed to settle before filtration. The fractionated pullulan was rinsed
106 two times with water and freeze-dried to yield a white cotton-like material. The pullulan
107 samples were sealed in poly(ethylene) bags under the exclusion of air and stored at 4°C.

108 *2.3 Preparation of pullulan-based formulations*

109 The lyophilized fractionated pullulan was found to have a moisture content of 2.5 wt-% after
110 storage for 4 weeks over P₂O₅ in a desiccator, as determined by thermogravimetric
111 measurements at a constant temperature of 120°C for 72 hours. The dried samples after such
112 a treatment have a moisture content lower than 1 wt-%. Samples were stored and irradiated as
113 such in sealed air-tight vials.

114 Hydro-methanolic blends were prepared by placing the desired amount of lyophilized
115 pullulan in a porcelain dish and by adding water over the entire volume, keeping the ratio
116 pullulan : water = 70 : 60 parts by weight. The hydrated pullulan formed a viscous paste after
117 conditioning at 50°C for around 30 min. The paste was allowed to cool to room temperature.
118 Methanol was then added dropwise and gently mixed manually, giving the final blend
119 composition of pullulan : water : methanol in 70 : 60 : 30 parts by weight, respectively. The

120 blends containing CA were prepared by adding the aromatic alcohol in small fractions (0.002
121 to 0.20 equivalents per mole of AGU) to prevent any macrophase separation. Each
122 formulation was prepared, treated and analyzed in duplicate.

123 *2.4 Irradiation conditions*

124 The electron beam accelerator used for irradiation was a 10 MeV Circe II industrial scale
125 accelerator (Linac Technology, France) operated at IONISOS, Chaumesnil, France. The
126 samples were sealed in glass vials in ambient air and irradiated with an average dose rate of 3
127 kGy s⁻¹ at 10, 25, 50, 75, 100 and 200 kGy doses, by applying 25 kGy per pass for doses of
128 25 kGy and above. Dose values were controlled by using calibrated calorimeters with an
129 accuracy of $\pm 2\%$.

130 *2.5 SEC analysis*

131 The size exclusion chromatography (SEC) system used for this study was based on a DMSO
132 and water mixture (20 : 80 v/v) as mobile phase containing 0.02 percent in weight (wt-%)
133 NaN₃, as an anti-bacterial agent and background electrolyte (308 mM). The sample solutions
134 were prepared by weighing 80 mg of formulation and adding 4 mL of the mobile phase
135 followed by heating at 60°C for 30 min. Complete solubility of the formulation in the mobile
136 phase resulted in a clear solution that was filtered using 0.45µm PTFE syringe filters before
137 injection into the SEC system. The separation was carried out on two Polargel columns M
138 and L kept in series (obtained from Varian- Agilent Technologies) behind a Polargel-specific
139 guard column (Varian-Agilent Technologies). The mobile phase flowing at a constant rate of
140 1 mL min⁻¹ was analyzed by two detectors in series, first a UV Absorbance detector (Jasco
141 UV-1575) set to 282 nm and then a differential refractive index (RI) detector (Precision
142 Instruments IOTA 2). Samples were injected with a 50 µL-volume using an auto-sampler
143 (Jasco AS-350) .

144 The calibration curve was established from the retention times based on RI detection of 9
145 pullulan standards of low dispersity ($\mathcal{D} \sim 1.05$) covering a broad MW range between 700 and
146 800000 g mol⁻¹ (Varian-Agilent Technologies). Every sample was analyzed by at least 3
147 injections for calculating the average MWs of the polymers.

148 **3. Results and discussion**

149 *3.1. Theoretical considerations*

150 To apply the formalisms developed independently by Charlesby and Pinner, and by Saito, to
151 the changes in molecular mass for a given type of polymer as a function of absorbed dose of
152 radiation, the treated polymer must comply with certain critical requirements that can be
153 summarized as follows: (i) the initial polymer structure should be topologically linear, (ii) the
154 events of scission and crosslinking should occur randomly along the chains and their extent
155 must directly be proportional to the dose, (iii) the number of monomer units affected by
156 chemical events should be considerably lower than the number-average degree of
157 polymerization (Saito, 1972; Charlesby, 1980, Moad & Winzor, 1998; Rosiak, 1998).

158 Depending on the initial MW distribution and on the relative importance of scission and
159 crosslink probabilities in the treated polymer, several relations have been derived from the
160 statistical models to correlate the radiation chemical yields $G(S)$ and $G(X)$ to the change of
161 MW distribution. If the starting polymer has a most probable MW distribution ($\bar{M}_w / \bar{M}_n = 2$),
162 the two models provide relatively simple equations relating to the dose-dependence of
163 quantities deduced from experimental data. If gelation occurs, the Charlesby-Pinner relation
164 (Equ. 1) predicts a linear variation of the quantity $(s + s^{0.5})$, where s is the sol fraction, with
165 the reciprocal value of the dose D (kGy), giving access to $G(S)$ and $G(X)$ values in mol J⁻¹ for
166 the polymer of initial weight-average MW, \bar{M}_w^0 expressed in g mol⁻¹.

167
$$s + \sqrt{s} = \frac{G(S)}{2G(X)} + \frac{1}{\bar{M}_w^0 G(X) D} \quad (1)$$

168 The second method is based on the evolution of polymer molecular masses, the reciprocal
 169 values of \bar{M}_n and \bar{M}_w being predicted to vary linearly with the applied dose D (kGy) and this
 170 relation, the $G(S)$ and $G(X)$ values in mol J⁻¹ as well as the initial molecular masses (\bar{M}_n^0 and
 171 \bar{M}_w^0) appearing as constants in equations 2 and 3, respectively.

172
$$G(S) - G(X) = \frac{1}{D} \left(\frac{1}{\bar{M}_n} - \frac{1}{\bar{M}_n^0} \right) \quad (2)$$

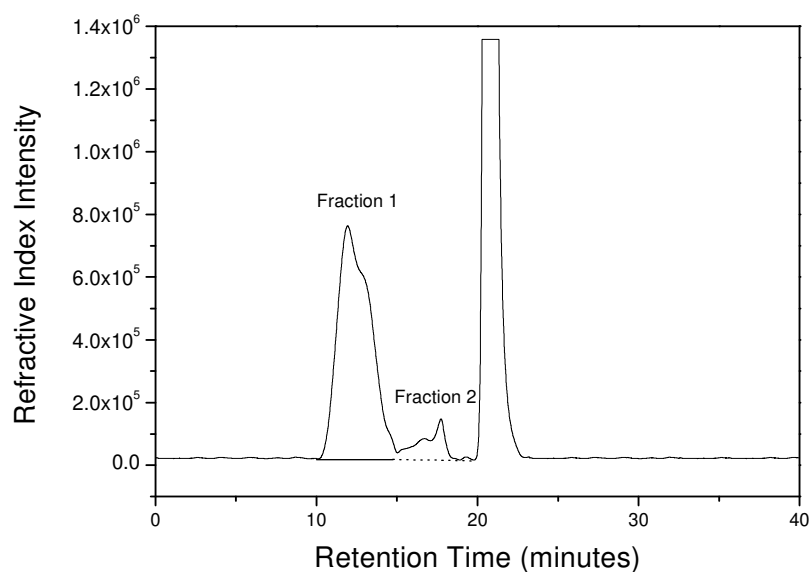
173
$$\frac{G(S)}{2} - 2G(X) = \frac{1}{D} \left(\frac{1}{\bar{M}_w} - \frac{1}{\bar{M}_w^0} \right) \quad (3)$$

174 In practice, if the corresponding plots of $(\bar{M}_n)^{-1}$ and $(\bar{M}_w)^{-1}$ as a function of dose are acceptably
 175 linear over a statistically relevant number of experiments, the values of the two slopes allow
 176 for the calculation of the $G(S)$ and $G(X)$ values for the system.

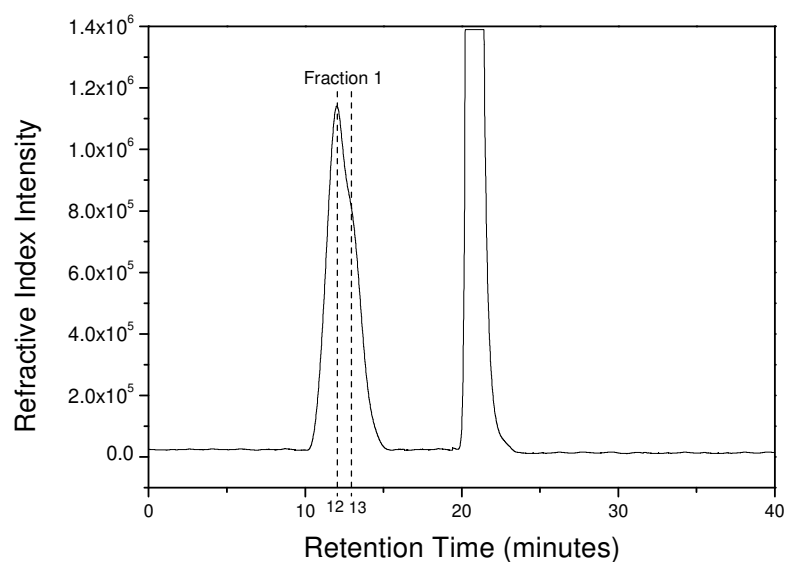
177 Despite certain limitations (Güven, 1990), size exclusion chromatography (SEC) is a
 178 particularly suitable method used for determining the changes in the average MWs of a
 179 polymer sample before the gel point, in the eventuality of predominant crosslinking. This
 180 analytical technique has been reported for the study of pullulan degradation by γ -irradiation
 181 (Shingel et al., 2000).

182 3.2 Fractionation of Pullulan

183 The commercially obtained pullulan is a multimodal and highly polydisperse sample with
 184 two well separated domains in the SEC-RI profile, one representing the larger molecular
 185 mass fraction ($\bar{M}_w \sim 250,000 \text{ g mol}^{-1}$) and the other a smaller molecular mass fraction ($\bar{M}_w \sim$
 186 $4,400 \text{ g mol}^{-1}$) (Fig. 1 a). Since the commercial sample did not satisfy the requirements of the
 187 statistical model, the separation of the larger molecular mass fraction of pullulan was
 188 performed to obtain a pullulan with random molecular weight distribution.



189 a)



190 b)

191 Fig. 1. SEC traces of the original (a) and of the fractionated pullulan (b) recorded with refractive index
 192 detection.

193
 194 Fractionation of the commercial pullulan sample was attempted using different methods of
 195 separation such as preparative chromatography and precipitation by the addition of poor
 196 solvents to the aqueous solution (methanol, ethanol, acetone, isopropanol, THF and
 197 acetonitrile). It was found that the solvent-selective precipitation method was faster and more
 198 efficient in terms of final dispersity $\mathcal{D} \sim 2$. The chromatographic separation method on a

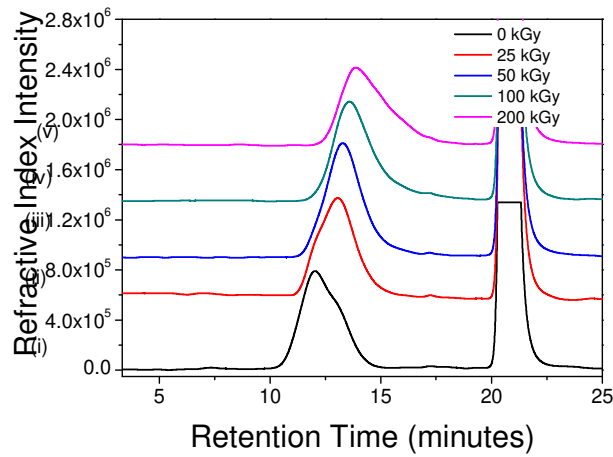
199 Sephadex column was shown to result in much narrower MW distribution, the dispersity \bar{D}
200 being typically between 1.2 and 1.5. Precipitation with methanol was found most convenient
201 and standardized over several trials with varying sample sizes. Good reproducibility was
202 achieved when the concentration of aqueous commercial pullulan solution was 5 % (w/v) and
203 the final methanol : water ratio remained close to but did not exceed the ratio of 6 : 4 (v/v).
204 After decantation and washing the precipitate with water, the fractionated pullulan samples
205 were freeze-dried. SEC analysis with the RI detection of this material (Fig. 1-b) showed a
206 single peak with a slight shoulder. The peak profile did not change for various pullulan
207 obtained from different batches, with retention times for the peak onset and the maximum
208 differing only by a few seconds. The average MWs for the entire peak of the fractionated
209 pullulan were $\bar{M}_w \sim 280,000 \text{ g mol}^{-1}$ and $\bar{M}_n \sim 130,000 \text{ g mol}^{-1}$, leading to a dispersity value
210 $\bar{D} \sim 2.15 \pm 0.03$. The small shoulder in the peak is considered of limited consequence in this
211 study because we are concerned with the evolution of the molecular mass of the entire peak,
212 independent of local variations of the distribution.

213 *3.3 Effect of ionizing radiation on $G(S)$ and $G(X)$ of pullulan treated in the solid state*

214 Besides the macromolecular effects of ionizing radiation to polysaccharide in general, and
215 polyglucans more specifically, the basic direct and indirect reactions that affect the monomer
216 units produce new functional groups on the polysaccharide that absorb in the UV region, such
217 as aldehydes, ketones, carboxylic acids, etc. (Phillips et al. 1966; Scherz, 1970; Ershov, 1998;
218 Shingel, 2002). Therefore, SEC analysis with double detection, UV absorption and
219 differential refractive index, brings additional information on the degree of modification in
220 constituting units as a function of pullulan chain length and as a function of dose.

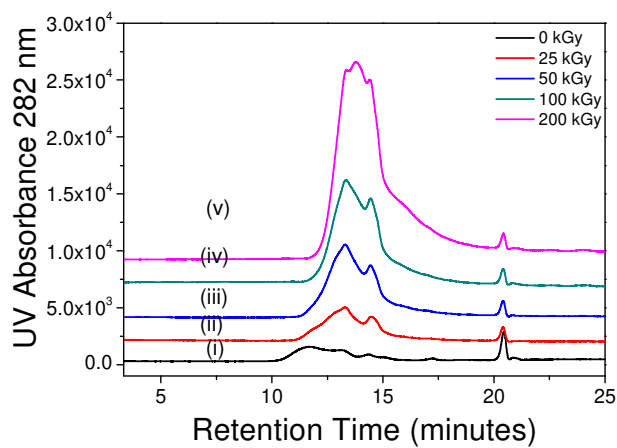
221 The chromatograms of Fig. 2 were recorded with differential refractive index detection of
222 fractionated pullulan irradiated in the dehydrated solid state for absorbed doses of radiation

223 ranging from 25 to 200 kGy. The evolution of the traces illustrates the modifications
224 occurring at the macromolecular and molecular levels.



225

a)



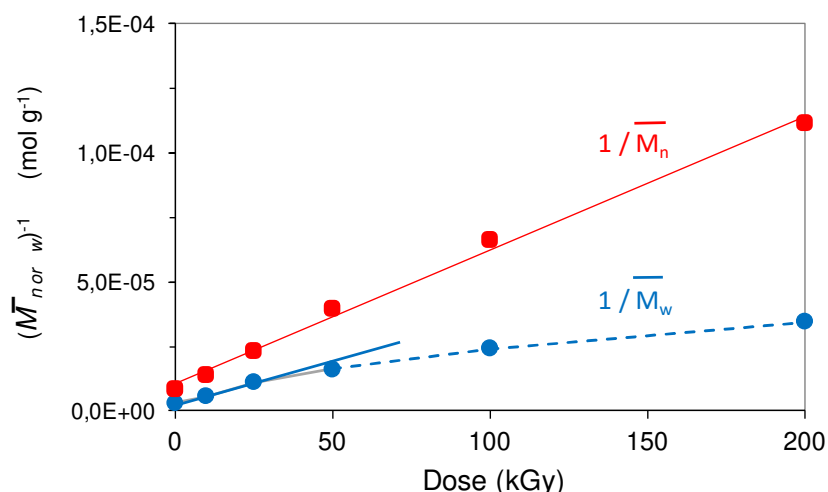
226

b)

227 Fig. 2. Evolution of SEC traces for the fractionated pullulan after EB irradiation as a dehydrated powder with
228 doses up to 200 kGy (0 kGy (i), 25 kGy (ii), 50 kGy (iii), 100 kGy (iv), 200 kGy (v)) recorded with differential
229 refractive index detection (a) and with UV detection at 282 nm (b).

230

231 The SEC traces obtained by differential refractive index detection confirmed the strong
232 propensity of pullulan to degrade, with a gradual shift toward longer retention times of the
233 whole distribution. The dose-dependence of the reciprocal values of \bar{M}_n and \bar{M}_w calculated
234 from the SEC traces is represented in the plots of Fig. 3.



235

236 Fig. 3. Evolution of reciprocal values of \bar{M}_n (■) and \bar{M}_w (●) calculated from SEC traces recorded with refractive
 237 index detection for samples of fractionated pullulan irradiated in the dry state with doses up to 200 kGy.

238

239 The reciprocal number-average mass \bar{M}_n^{-1} varies linearly across the whole range of doses
 240 explored for these experiments, following the predicted behavior expressed in equation 2,
 241 whereas the linearity of the reciprocal weight-average mass \bar{M}_w^{-1} is lost for absorbed radiation
 242 doses higher than 40-50 kGy. The explored dose range, up to 200 kGy, is quite high for a
 243 polysaccharide. The SEC traces obtained by UV detection at 282 nm (Fig. 2-b) indeed reveal
 244 the concomitant formation of chromophores, likely carbonyl groups, that are known to result
 245 from the rearrangement of the initial free radicals generated by direct radiolysis of the
 246 polysaccharide, and also by their subsequent oxidation in the presence of air (Ershov, 1998;
 247 Bhat et al., 2014).

248 This confirms that the initial structure of pullulan has been profoundly modified. Its reactivity
 249 as well as its macromolecular properties should then be affected accordingly. Such chemical
 250 changes may explain the deviation from linearity, since the basic hypotheses of the statistical
 251 model are no longer fulfilled.

252 The linear domains of the plots covering the low dose range (from 0 to about 50 kGy) were
 253 then used to calculate the $G(S)$ and $G(X)$ values, by combining equations 2 and 3. The results

254 in entry a of Table 1 show that the slopes are calculated in the domain from 0 to 25 kGy with
 255 acceptable correlation coefficients, yielding a $G(X)$ value close to zero and $G(S)$ of about 0.6
 256 $\mu\text{mol J}^{-1}$.

257 Table 1. Determination of the radiation chemical yields $G(S)$ and $G(X)$ using SEC data measured for
 258 fractionated pullulan samples irradiated in the solid state.

Entry	Pullulan sample	$(\bar{M}_w)^{-1}$ versus dose		$(\bar{M}_n)^{-1}$ versus dose		$G(S)$ ($\mu\text{mol J}^{-1}$)	$G(X)$ ($\mu\text{mol J}^{-1}$)
		$m_w^{\text{a)}$	$R_w^{\text{b)}$	$m_n^{\text{a)}$	$R_n^{\text{b)}$		
a	Powder dehydrated over P_2O_5	$5.8 \cdot 10^{-7}$	0.996	$2.7 \cdot 10^{-7}$	0.997	0.59	0.015
b	Powder with a moisture content of 2.5 wt-%	$4.0 \cdot 10^{-7}$	0.999	$1.8 \cdot 10^{-7}$	0.994	0.41	0.015

259 ^{a)} m_w and m_n represent the slopes of the linear regression for the plots of Fig. 3 and 4.

260 ^{b)} R_w and R_n are the coefficients of correlation of the corresponding linear regressions.

261

262 Similar experiments were conducted with fractionated pullulan irradiated in the solid state
 263 with a moisture content of 2.5 wt-%. Not surprisingly, the $G(X)$ value is again close to zero
 264 for the neat polysaccharide (entry b of Table 1), but the $G(S)$ value of $0.41 \mu\text{mol J}^{-1}$, is
 265 somewhat lower than for the dehydrated sample. Similar protective effects by moisture has
 266 been reported in the early studies of the effects of radiation on polysaccharides (Chapiro,
 267 1962), amylose (Ehrenberg et al., 1957) and cellulose derivatives (Leavitt, 1961; Leavitt,
 268 1962).

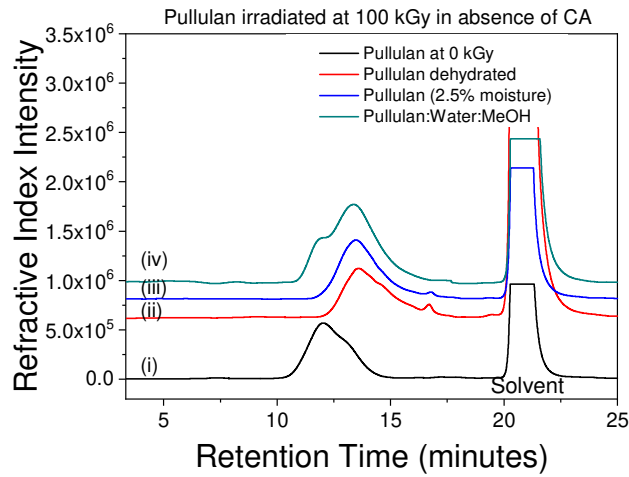
269 Few articles report reliable data using the theoretical models to quantify the behavior of
 270 polysaccharides under radiation. Among the glucose-based polysaccharides, amylose was
 271 shown to degrade with $G(S)$ values between 0.5 and $1.35 \mu\text{mol J}^{-1}$ (Ehrenberg, 1957) and
 272 cellulose with a $G(S)$ value of $0.56 \mu\text{mol J}^{-1}$ (Ershov, 1998). The $G(S)$ values reported for
 273 other polysaccharides, based on modified glucose units or other types of sugar units carrying
 274 ionic groups, were found to be between 0.09 and $2.1 \mu\text{mol J}^{-1}$ for chitosan samples irradiated
 275 in the solid state, depending on their degree of deacetylation (Ulanski & Rosiak, 1992;

276 Taskin et al., 2014), between 0.15 and 0.25 for carboxymethyl cellulose (Fei et al., 2000), and
277 from 0.1 to 0.4 $\mu\text{mol J}^{-1}$ for various sodium alginates (Sen et al., 2014). Despite the
278 differences in the conditions for irradiation treatments and in the analytical methods that were
279 used, the scissioning yield that we report with a $G(S)$ value of 0.59 for dry pullulan compares
280 well with values determined for amylose and cellulose, the linear polysaccharides showing
281 the closest structural similarities with pullulan.

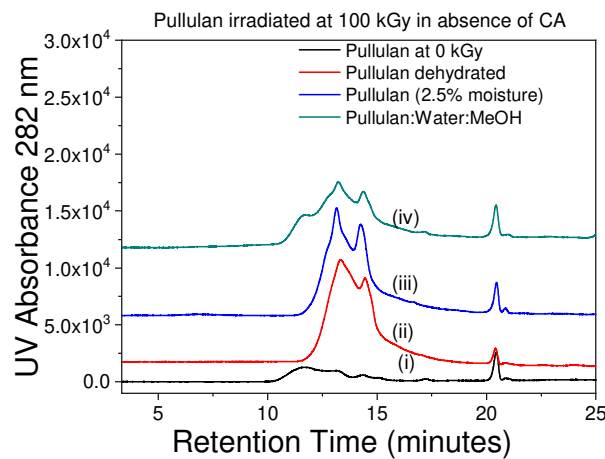
282 Strictly speaking, the next coming part of our work is somehow on the borderline of the basic
283 frame of the theoretical models describing the radiation behavior of irradiated polymers. We
284 wanted to apply this approach to study the influence of minor amounts of additives in protic
285 formulations including water and methanol. This was motivated by our previous studies on
286 the influence of an aromatic alcohol, CA, and on glycerol in the maltodextrine-based blends,
287 these additives having shown a strong impact on the gelation of maltodextrine blends
288 (Lepifre et al., 2004; Khandal et al. 2012, Khandal et al., 2013; Khandal et al., 2015). Various
289 formulations containing the same pullulan at the same concentration were thus considered as
290 a whole, to examine the influence of the composition in the additives on the apparent
291 radiation chemical yields $G_{app}(S)$ and $G_{app}(X)$ values for pullulan irradiated in complex
292 blends. The influence of the hydro-methanolic dispersing medium was examined at first by
293 comparing pullulan-based formulations having a paste-like consistency due to the use a
294 limited amount of solvents. The relatively high concentration of pullulan in these blends not
295 only favors crosslinking by enhancing the occurrence of intermolecular coupling between
296 chains, but also allows to achieve an acceptable degree of dispersion of the components
297 within the slurry, even in the presence of a relatively hydrophobic additive such as CA (see
298 section 3.5).

299 *3.4 $G(S)$ and $G(X)$ of pullulan in hydro-methanolic blends irradiated in absence of CA*

300 The SEC traces recorded with differential refractive index and UV (282 nm) detection of
301 pullulan and pullulan-based slurries after irradiation at 100 kGy are shown in Fig. 4-a and
302 Fig. 4-b, respectively.



303 a)



304 b)

305 Fig. 4. SEC traces with differential refractive index (a) and UV detection at 282 nm (b) for pullulan unirradiated
306 (i), and irradiated at a 100 kGy dose in the dry state (ii), with 2.5 wt-% of moisture (iii), in paste-like blend of
307 composition pullulan : water : methanol (70/60/30 parts by weight) (iv).
308

309 In comparison with the unirradiated pullulan, the hydro-methanolic blend reveals an overall
310 increase in the retention times for the polysaccharide component, indicating a marked
311 decrease in the chain length resulting from the radiation treatment. However, the profiles
312 show a lower level of chain scission than for pullulan irradiated in the dry state or with a low
313 moisture content. Also, the presence of shoulder on the high-MW side of the main peak

314 suggests the occurrence of some intermolecular coupling reactions, whereas the formation of
 315 chromophores absorbing at 282 nm, appreciated from the area of chromatograms recorded for
 316 samples prepared with the same concentration in pullulan, is somewhat lower for the paste-
 317 like hydro-methanolic blend.

318 The evolutions of the $(\bar{M}_w)^{-1}$ and $(\bar{M}_n)^{-1}$ values for pullulan calculated from the differential
 319 refractive index chromatograms for all the blends were plotted against the absorbed dose of
 320 radiation. For doses in the lower range, up to 50 kGy for $(\bar{M}_w)^{-1}$, and up to 100 kGy for $(\bar{M}_n)^{-1}$,
 321 the plots of Fig. 5 show an acceptable degree of linearity with correlation coefficients R equal
 322 to 0.996 and 0.999, respectively, allowing for the determination of apparent radiation
 323 chemical yields. To take into account, both from energetic and chemical viewpoints, the
 324 indirect chemical effects caused by radiation within the fluid medium onto the pullulan
 325 component, corrected chemical yields $G(S)_{corr}$ and $G(X)_{corr}$ are calculated by introducing the
 326 fraction in weight (f_w) of pullulan within the hydro-methanolic blend samples, using
 327 equations 4 and 5.

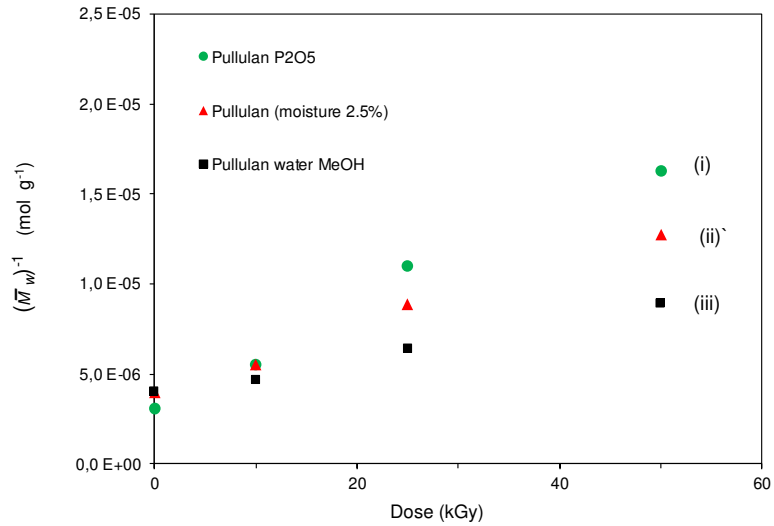
$$328 \quad G(S)_{corr} - G(X)_{corr} = \frac{f_w}{D} \left(\frac{1}{\bar{M}_n} - \frac{1}{\bar{M}_n^0} \right) \quad (4)$$

$$329 \quad \frac{G(S)_{corr}}{2} - 2G(X)_{corr} = \frac{f_w}{D} \left(\frac{1}{\bar{M}_w} - \frac{1}{\bar{M}_w^0} \right) \quad (5)$$

330 By this way, the number of events considered for each corrected G value are ratioed to the
 331 total energy deposited in the whole sample. In the present case, all blend samples include
 332 pullulan with the same weight-fraction, $f_w = 0.4375$, yielding the corrected values $G(S)_{corr}$
 333 $0.134 \mu\text{mol J}^{-1}$ and $G(X)_{corr} 0.0117 \mu\text{mol J}^{-1}$.

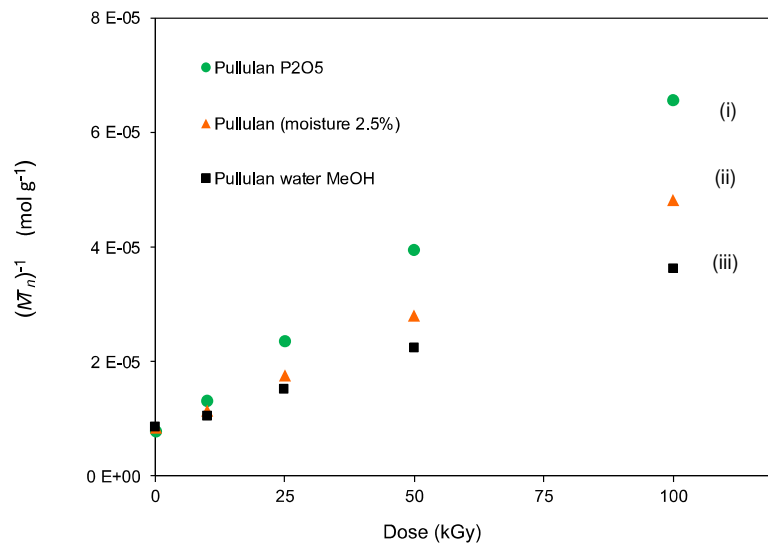
334 The deviation from linearity observed for higher doses can either originate from unsatisfied
 335 assumptions on the basic hypotheses required by Saito's model, or by a lack of accuracy of
 336 the MW calculated from the SEC traces for pullulan chains strongly modified by the
 337 radiation treatment, particularly if interchain branching takes place.

338 The $G(S)/G(X)$ ratio determined for each blend sample is independent of the concentration in
 339 polymer. This ratio is interesting from a practical viewpoint, since the statistical models
 340 mentioned in the introduction predict that the irradiated polymer can undergo gelation if
 341 $G(S)/G(X) < 4$, or will not form a tridimensional network at any dose if $G(S)/G(X) > 4$.



342 a)

b)



343

344 Fig. 5. Plots showing the linear part of the variations of $(\bar{M}_w)^{-1}$ (a) and $(\bar{M}_n)^{-1}$ (b) as a function of absorbed dose
 345 of radiation across the lower range, for pullulan dehydrated (i), pullulan with a moisture content of 2.5 wt-% (ii),
 346 hydro-methanolic paste of pullulan (iii).

347

348 Compared to pullulan irradiated in the dry solid state, with $G(S) = 0.59$, the $G(S)$ values
 349 determined for the paste-like hydro-methanolic blend is lower by a factor approximately 4,

350 with $G(S)_{corr}$ close to 0.15 for pullulan in water : methanol, whereas the $G(X)_{corr}$ value
351 appears to be much less affected.

352 The direct and indirect effects of radiation-induced processes on pullulan are complex in such
353 concentrated and multicomponent blends. Water radiolysis occurring in these blends
354 generates various active species (Hart, 1965; Le Caër, 2011), among which HO^\bullet radicals are
355 known to efficiently abstract hydrogen atoms from the polysaccharide and induce the
356 formation of C-centered free radicals at different positions of the AGU moieties (Von
357 Sonntag & Schuchmann, 2001). Alcohols act as scavengers of HO^\bullet radicals (Baugh et al.,
358 1982; Buxton et al., 1988), therefore being expected to decrease the indirect radiolytic effects
359 on both scission and crosslinking compared to simpler pullulan-water blends. Accordingly,
360 the $G(X)$ and $G(S)$ values that were determined from the linear segments of the plots of Fig. 5
361 are significantly lower when an alcohol is present in the blend.

362 In addition to the chemical effects on the radiolytic and free radical processes, the hydro-
363 methanolic medium has physical effects by enhancing chain mobility that would likely
364 increase the possibility of interchain coupling between the macroradicals. Compared to the
365 solid state and to dilute solution, paste-like blend samples offer much favorable conditions
366 for ensuring gel formation (Wach et al, 2003; Yoshii et al., 2003).

367 At higher dose levels, the loss of linearity in the plots of the reciprocal MWs for pullulan in
368 hydro-methanolic blends reveals a change in behavior, with stronger competition by
369 intermolecular coupling. There is an inversion in the curves, indicating an overall increase of
370 \bar{M}_n and \bar{M}_w values (Fig. 6 a-b, curves (iv)).

371

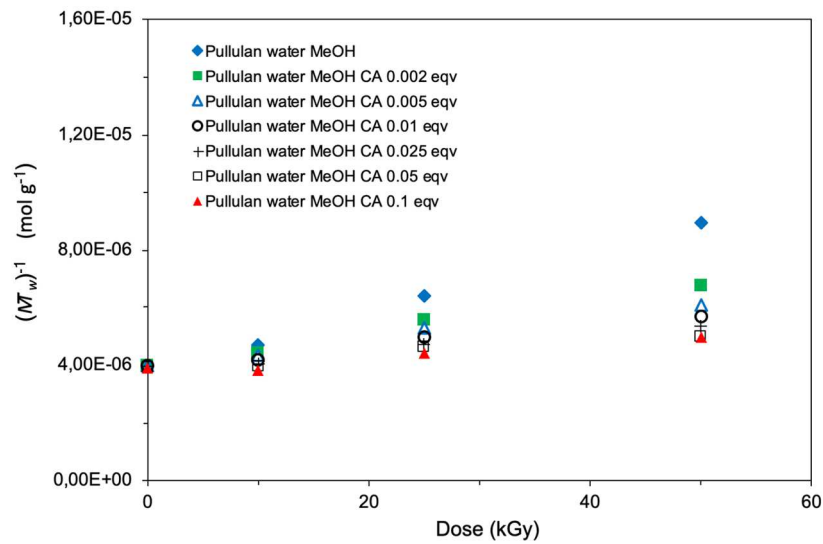
372 *3.5 $G(S)$ and $G(X)$ of pullulan in hydro-methanolic blends irradiated in presence of CA*

373 A remarkable feature of the pullulan blends including CA additive in the water-methanol
374 solvent is the drastic increase in viscosity or even the occurrence of gelation when they are

375 treated at a dose of 100 kGy or higher. The most interesting formulations in that respect were
376 the water-methanol blends containing 0.1 and 0.2 equivalent of CA per mole of AGU that
377 formed considerable amounts of gel when irradiated above 100 kGy, whereas the samples
378 with lower CA content either showed an increase in viscosity or some signs of incipient gel
379 formation.

380 To gain a better understanding of the effect of CA on the competition between radiation-
381 induced scission and interchain coupling for pullulan in hydro-methanolic media, we have
382 monitored the changes of MW distribution in blends with CA contents varying between
383 0.002 to 0.2 equivalents per mole of AGU from pullulan using the same analytical approach
384 as was the one described in the previous section. The blends were prepared accordingly with
385 CA concentration between 5.4 and 270.0 mmol kg⁻¹ and a constant AGU concentration of 2.7
386 mol kg⁻¹.

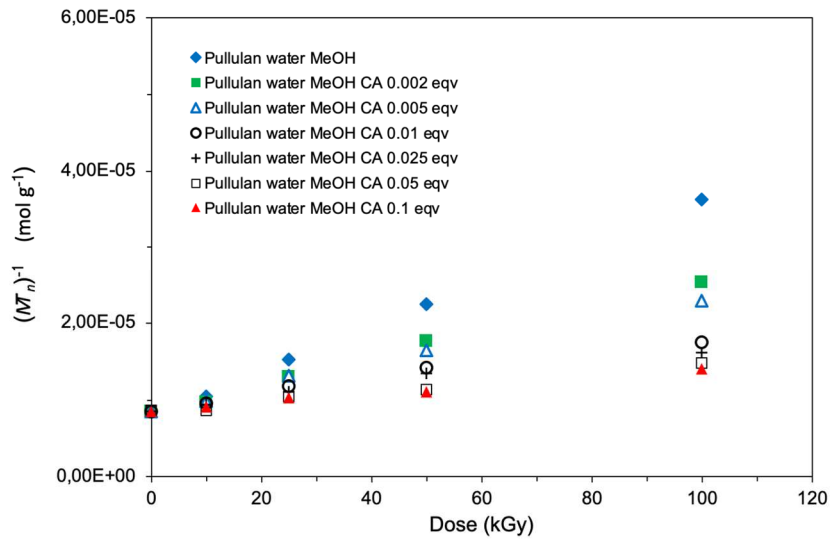
387



388

389

390



391

b)

392 Fig. 6. Evolution of (a) $(\bar{M}_w)^{-1}$, and (b) $(\bar{M}_n)^{-1}$ as a function of dose for pullulan blends in hydro-methanolic (70 :
393 60 : 30 parts by weight) including various amounts of CA.

394

395 Table 2. $G(S)$ and $G(X)$ values for pullulan in hydro-methanolic blends as a function of dose for various CA
396 contents, based on the slopes of $(\bar{M}_w)^{-1}$ and $(\bar{M}_n)^{-1}$ in Fig. 7.

Pullulan in in water : methanol 70 : 60 : 30 (parts by weight)	Slope $(\bar{M}_w)^{-1}$ vs D		Slope $(\bar{M}_n)^{-1}$ vs D		$G(S)_{corr}$ ($\mu\text{mol J}^{-1}$)	$G(X)_{corr}$ ($\mu\text{mol J}^{-1}$)
	m_w	R_w	m_n	R_n		
no CA	$1.01 \cdot 10^{-7}$	0.996	$2.81 \cdot 10^{-7}$	0.999	0.1340	0.0115
CA 0.002 equiv. / 5.4 mmol kg^{-1}	$0.571 \cdot 10^{-7}$	0.990	$1.70 \cdot 10^{-7}$	0.996	0.0826	0.0083
CA 0.005 equiv. / $13.5 \text{ mmol kg}^{-1}$	$0.43 \cdot 10^{-7}$	0.996	$1.44 \cdot 10^{-7}$	0.973	0.0715	0.0085
CA 0.01 equiv. / $27.0 \text{ mmol kg}^{-1}$	$0.36 \cdot 10^{-7}$	0.978	$0.89 \cdot 10^{-7}$	0.977	0.0416	0.0026
CA 0.025 equiv. / $67.5 \text{ mmol kg}^{-1}$	$0.29 \cdot 10^{-7}$	0.977	$0.76 \cdot 10^{-7}$	0.975	0.0360	0.0026
CA 0.05 equiv. / $135.0 \text{ mmol kg}^{-1}$	$0.23 \cdot 10^{-7}$	0.924	$0.54 \cdot 10^{-7}$	0.990	0.0295	0.0022
CA 0.1 equiv. / $270.0 \text{ mmol kg}^{-1}$	$0.23 \cdot 10^{-7}$	0.921	$0.62 \cdot 10^{-7}$	0.983	0.0249	0.0012

397

a) m_w and m_n represent the slopes $\text{mol g}^{-1} \text{kGy}^{-1}$ of the linear regression for the plots of Fig. 9.

398

b) R_w and R_n are the coefficients of correlation of the corresponding linear regressions.

399

400 Compared to the profiles recorded with irradiated samples that did not contain the aromatic
401 additive, the first qualitative observation is the mitigated trend for chain degradation and the

402 gradual emergence of bimodality in the MW distribution, with the persistence of a fraction
403 with longer polymer chain. The average molecular masses were calculated for each of these
404 formulations (see Supporting information) to plot the dose-dependence of $(\bar{M}_w)^{-1}$ and $(\bar{M}_n)^{-1}$
405 values for a large series of blends with various amounts of CA additive. The linearity of the
406 plots was satisfactory for doses up to 50 kGy (Fig. 6).

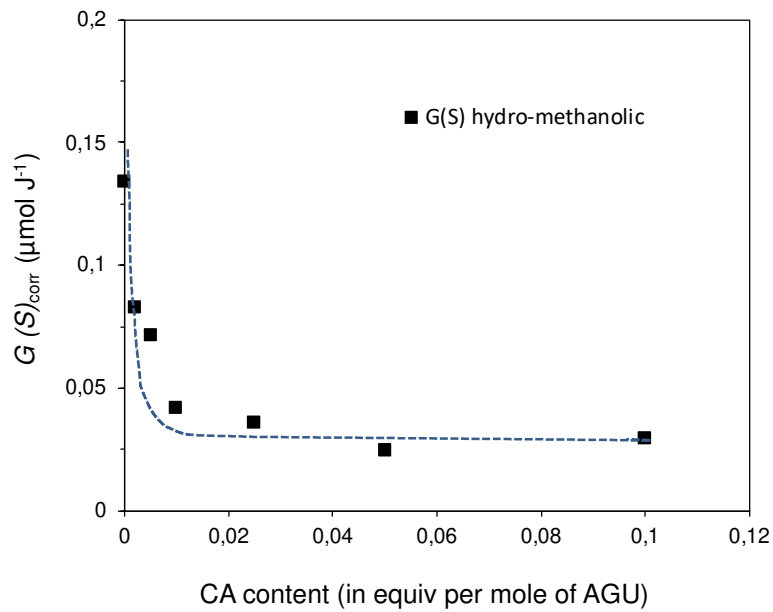
407 In both cases, the points corresponding to the 10 kGy dose appear slightly above the
408 theoretical straight line. We **assume** that the presence of trace amounts of oxygen affects the
409 radiolytic reactions at low doses, until the complete consumption of the dissolved O₂. The
410 dependence over the dose range up to 50 kGy was considered more representative of the
411 response of the system to the radiolytic treatment. The participation of CA to radiation-
412 induced reactions could also affect the composition of the blends upon irradiation. However,
413 we have determined by ¹H NMR spectroscopy that less than 40 mol-% of CA was modified
414 by treatment at 200 kGy for the hydro-methanolic pullulan blend containing 0.2 **equivalent** of
415 the aromatic alcohol (see Supporting information). We can therefore assume that the
416 concentration of CA was approximately constant within all the samples during the treatment
417 by much lower doses (up to 50 kGy).

418 The $G(S)_{corr}$ and $G(X)_{corr}$ values calculated for pullulan in hydro-methanolic blends are
419 reported in Table 1 together with the correlation coefficient of **associated linear variations**.

420 The corresponding plots of Fig. 7-a and 7-b clearly show the drastic decline in $G(S)$ and $G(X)$
421 for amounts as small as 0.002 equivalents of CA per mole of AGU in the blends,
422 corresponding to a concentration of 5.4 mmol per kg of the blend. This decrease in G -values
423 suggests a strong reduction of the number of free radicals generated by direct or indirect
424 radiolysis on pullulan chains.

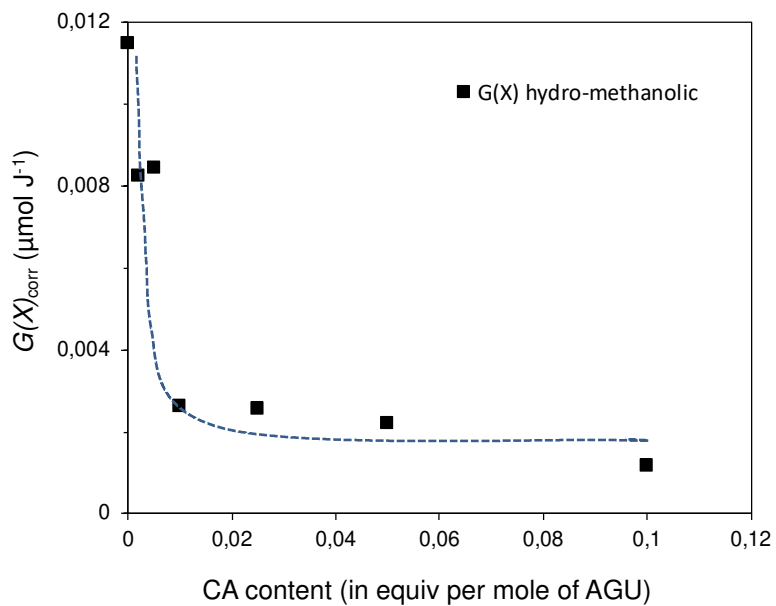
425

426



427

a)



428

b)

429 Fig. 7. Comparison of the (a) $G(S)$ and (b) $G(X)$ values for pullulan : water : methanol (70 : 60 : 30 parts by
430 weight) formulations containing varying amounts of CA and irradiated in the dose range of 0 to 60 kGy.

431

432 The presence of aromatic compounds in irradiated blends, as well as the presence of aromatic
433 moieties in irradiated organic compounds, are known to mitigate radiolytic processes,
434 particularly the formation of molecular hydrogen (Soebianto et al., 1992; Soebianto et al.,
435 1993; Tabuse et al., 2001; La Verne & Dowling-Medley, 2015). Similar protective effects

436 due to energy and charge transfer to aromatic additives seem to operate here, affecting with
437 approximately the same attenuation factor both radiation chemical yields, therefore with
438 limited impact on the $G(S) / G(X)$ ratio in the low dose range (see Table 2).

439

440 **4. Conclusion**

441

442 The radiation chemical yields of scission and crosslinking for pullulan submitted to electron
443 beam radiation in the dry state ($G(S) = 0.59$, $G(X) < 0.02$) complement the available sets of
444 data on the radiolysis of polysaccharides. The method based on the monitoring of changes in
445 **molecular weight** by SEC confirmed the influence of small amounts of moisture on both G
446 values.

447 Our study emphasizes the effect of hydro-methanolic media on paste-like pullulan blends,
448 showing that indirect effects of water radiolysis are overcome by alcohol-mediated
449 scavenging of HO^\bullet radicals, resulting in a decrease of $G(S)$. Introducing cinnamyl alcohol in
450 the hydro-methanolic pullulan blends further induces a strong protective effect with a
451 noticeable impact likely due to energy and / or electron transfer to the multifunctional
452 aromatic additive. A significant influence of CA was observed at concentrations as low as 5
453 mmol kg^{-1} in the blend.

454 In **another** component of this study, **we have analyzed by** MALDI-ToF mass spectrometry
455 and ^1H NMR spectroscopy, the modification occurring **with** pullulan and maltotriose. The
456 Charlesby-Pinner model based **on the gel fraction in irradiated pullulan** provides access to the
457 $G(S)$ and $G(X)$ values determined for the various blend compositions discussed here, but in
458 another dose range beyond the gel point. Structural analysis of modified pullulan and other
459 low molar mass model compounds **bring** additional information on the participation of CA to
460 the radiation-induced gelation that we observed in the higher dose range for blends
461 containing the aromatic additive at concentrations above 250 mmol kg^{-1} .

462 From a practical standpoint, the protective effect against main chain scissions demonstrated
463 in this report opens a new perspective for the radiation treatment of polysaccharides which is
464 generally considered to be strongly limited by the inherent sensitivity of this class of natural
465 polymers to radiolytic degradation. The influence of other natural aromatic compounds, such
466 as coumaric and ferulic acids, or of glycerol, as an alternative to methanol in the hydro-
467 alcoholic blends, are under investigation.

468

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475

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Highlights (3 to 5 bullet points - maximum 85 characters each)

- Pullulan was studied as a model for starch irradiated in dry or paste-like states
- Radiolytic yields for scission and cross-linking were determined using SEC data
- Cinnamyl alcohol exerts a radiation-protective effect even at low concentration
- Gelation is observed at high dose for cinnamyl alcohol exceeding 130 mmol kg⁻¹

